AUTOHYDROGENOTROPHIC PERCHLORATE REDUCTION

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Introduction

Perchlorate (ClO_4^-), an inorganic water contaminant, has recently been detected in surface waters or groundwaters in California, Nevada, Utah, Arkansas, and several other states. Perchlorate is highly stable when dissolved in water and is not removed by conventional water treatment processes. Therefore, its presence in surface or groundwater sources can pose a significant challenge to treatment facilities. Physical processes such as reverse osmosis and ion exchange can remove perchlorate; however, these methods are typically expensive and create perchlorate-containing waste streams that require subsequent treatment or disposal (Urbansky, 1998; USEPA, 1998). Biological degradation holds promise to be a more cost-effective treatment approach.

Researchers have long known of the existence of chlorate and perchlorate-reducing bacteria. Such bacteria reduce perchlorate and chlorate to the innocuous chloride ion (Cl^-) in a process providing energy for growth. Perchlorate-reducing bacteria appear to be ubiquitous in the natural environment, and many, but not all, are facultative anaerobes and denitrifiers (Bryan, 1954; Van Ginkel et al., 1995 Logan, 1998; Coates et al., 1999, Michaelidou et al., 1999). Biological treatment of high-strength chlorate or perchlorate wastewaters has been demonstrated (Malmqvist et al. 1992; Attaway and Smith, 1993; Attaway et al, 1994; Korenkov, et al., 1976; Wallace et al., 1998; Logan and Kim, 1998). However, several factors make such systems inappropriate for treating contaminated groundwaters: First, perchlorate is typically present at less than 100 μg/L levels, as opposed to gram per liter levels in some wastewaters, and it is not known if this concentration is sufficient to select for a perchlorate-reducing culture. Second, nitrate is present at levels typically greater than 1 mgN/L – more than one order of magnitude greater than perchlorate - and nitrate appears to have an inhibitory effect on perchlorate reduction. Third, the process requires the addition of a suitable electron donor at doses sufficient to achieve the reduction of nitrate and perchlorate. Methanol and ethanol are organic electron donors that have been used for perchlorate wastewater treatment systems. However, both compounds are alcohols that are federally regulated, and methanol has acute health risks.

Biological processes more appropriate for the drinking-water situation are needed. A critical issue is the choice of the electron donor substrate to fuel perchlorate reduction. Ethanol and

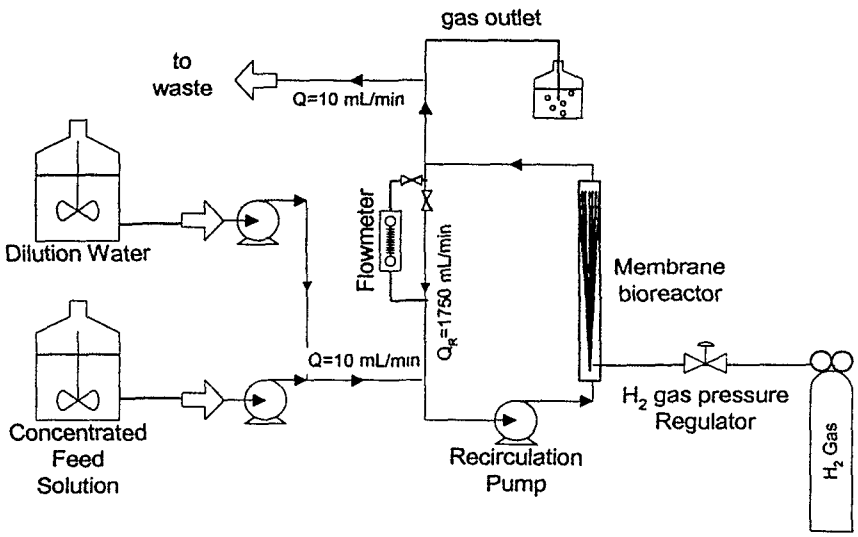
methanol present serious drawbacks. Other organic donors, such as acetate, overcome the toxicity and regulatory problems but still require addition of an easily biodegradable molecule that can cause biological instability, which creates regrowth potential in the distribution system.

Hydrogen also can serve as an electron donor and appears to be a particularly desirable choice, as it presents no toxicity, is inexpensive, and is sparsely soluble in water, so that it produces little regrowth potential in distributions systems. A disadvantage is that a hydrogen atmosphere in the presence of oxygen can create a potentially explosive atmosphere. Here, we report on the first stages of an experimental program to evaluate fundamentals of autohydrogenotrophic perchlorate reduction, or perchlorate reduction carried out by hydrogen-oxidizing autotrophic bacteria.

Methods

A completely mixed, autohydrogenotrophic (i.e., autotrophic, hydrogen-oxidizing) biofilm reactor was used to investigate potential kinetic mechanisms among hydrogen, perchlorate, and nitrate. The reactor uses a novel configuration (Lee, 1999) that improves operational safety by avoiding a hydrogen atmosphere. The system consists of a bundle of hollow-fiber membranes inside a PVC pipe shell (see **Figure 1**). Pure hydrogen is supplied to the inside of the hollow fibers through a manifold at the base, and hydrogen diffuses through the fiber material into the bulk water circulating in the PVC pipe. Therefore, only dissolved hydrogen is present in the reactor. When suitable electron acceptors are present in the circulating water, an autotrophic biofilm forms on the surface of the fibers. This configuration is highly efficient and is advantageous because hydrogen is delivered directly to the biofilm; because no bubble formation occurs, hydrogen wastage is minimized, and an explosive atmosphere is prevented.

Figure 1



Autohydrogenotrophic Biofilm Membrane Reactor

The reactor is supplied with a mixture of concentrated feed stock diluted with argon-sparged water. The water sparging minimizes the dissolved oxygen concentration in the feed. Water inside the reactor is recirculated at a rate of 1,750 mL/min to create well-mixed conditions. This minimizes substrate concentration gradients along the length of the biofilm reactor (i.e., with little change in concentration between the reactor inlet and outlet), allowing for an even distribution of biomass and facilitating the analysis of reactor performance during laboratory experiments. The fibers are made of a composite material consisting of a dense, 0.5-μm polyurethane core encased in microporous polyethylene with a 280-μm outside diameter. Overall mass transfer is limited by the diffusion through the dense, polyurethane core, which prevents the formation of hydrogen gas bubbles in the surrounding liquid phase. The reactor detention time is approximately 40 minutes.

The hydrogen reactor was initially seeded with a mixed autohydrogenotrophic culture from a previous denitrification study. Two sets of experiments were performed. The first served as a Screening Experiment, conducted in order to assess the reactor's potential for perchlorate reduction. The reactor was allowed to reach steady state with a feed solution with 1 mg/L perchlorate, 5 mgN/L nitrate, a phosphate buffer, and a hydrogen supply pressure of 7 psi. The second set included a series of short-term, or pseudo-steady-state, experiments performed to explore perchlorate reduction mechanisms. This second set was the first stage of a series of Mechanisms Experiments. Pseudo-steady-state conditions were achieved by performing experiments in a time frame long enough to reach hydraulic steady-state, but short enough to prevent appreciable changes in biomass. Each short-term experiment involved changing a single parameter and observing the reactor's immediate response. Prior to commencing the second-set experiments, the hollow-fiber membrane was removed and washed to remove excess biomass. A new steady-state biofilm was developed based on the same feed solution as the previous, steady-state experiment (1 mg/L perchlorate, 5 mgN/L nitrate), but with hydrogen pressure for the hollow fiber membrane reduced to 2.5 psi. The lower hydrogen pressure was selected to minimize the hydrogen concentration in the bulk liquid and thereby minimize biofilm growth on the reactor walls. In all experiments, perchlorate, chlorate, chlorite, chloride, nitrate, and nitrite were measured by ion chromatography following EPA Method 300.

Results

Steady-State

During the Screening Experiment, the nitrate removal was 96 percent and perchlorate removal was 39 percent one day after perchlorate feed was commenced. After one week, nitrate reduction increased to 99 percent, and perchlorate reduction increased to 90 percent. After 18 days, nitrate reduction had stabilized at 99 percent and perchlorate reduction increased to 95 percent. The effluent hydrogen concentration was approximately 0.5 mg/L. For the Mechanisms Experiments, steady-state nitrate reduction was 99 percent, perchlorate reduction was around 30 percent, and effluent dissolved hydrogen was 0.07 mg/L.

Short-Term, Pseudo-Steady-State Experiments

Hydrogen was the variable in the first set of short-term experiments. The applied hydrogen pressure was set to 1.5 psi, 2.5 psi, 4 psi, or 5.5 psi. Results are shown in **Figure 2,** both in terms of percentage removals and concentrations. At 1.5 psi, the effluent residual hydrogen was 11 µg/L, providing partial (86 percent) reduction of nitrate to 30 µgN/L. Under these conditions, the perchlorate reduction was only 5 percent. At 2.5 psi applied pressure, the effluent residual hydrogen was 37 µg/L and denitrification reached its maximum removal of 99 percent. Nitrate removals did not increase further at higher hydrogen pressures. At 2.5 psi, perchlorate reduction increased to 15 percent. At an applied pressure of 4 psi, with effluent residual hydrogen of 310 µg/L, perchlorate reached its maximum removal of 35 percent.

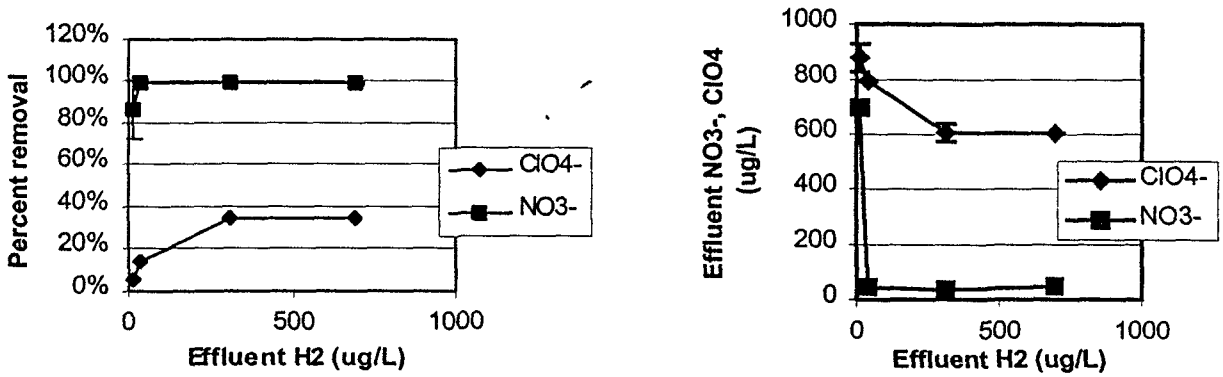


Figure 2
Short Term Experiment 1 – Hydrogen Partial Pressure is the Variable

Perchlorate was the variable in the second set of short-term experiments. Influent perchlorate concentrations were set at 0, 1000, 5000, or 25000 µg/L, with applied hydrogen and influent nitrate at their steady-state values of 2.5 psi and 5 mgN/L, respectively. Results are shown in **Figure 3**. The perchlorate concentration had no effect on nitrate reduction. Perchlorate removal declined significantly with the increased perchlorate load. The effluent hydrogen concentration varied from 30 to 50 µg/L (not shown).

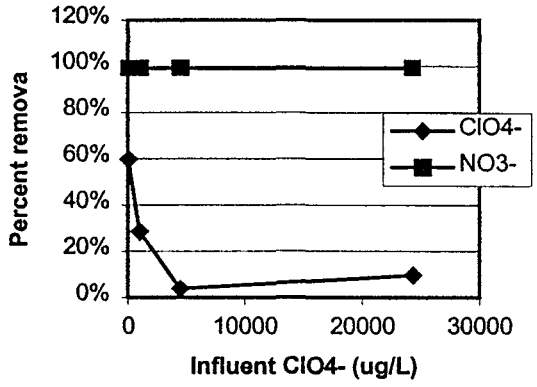


Figure 3
Short Term Experiment 2 – Influent Perchlorate is the Variable

Nitrate was the variable in the third set of short-term experiments. Nitrate influent concentrations were set at 0.05, 2.5, 10, and 15 mgN/L, with hydrogen pressure set at 5 psi and perchlorate at the steady-state concentration of 1 mg/L. Results are shown in **Figure 4**. With zero nitrate in the influent and effluent, perchlorate reduction increased to 57 percent. However, with effluent nitrate concentrations above 14 µgN/L, perchlorate reduction decreased to 25 to 30 percent.

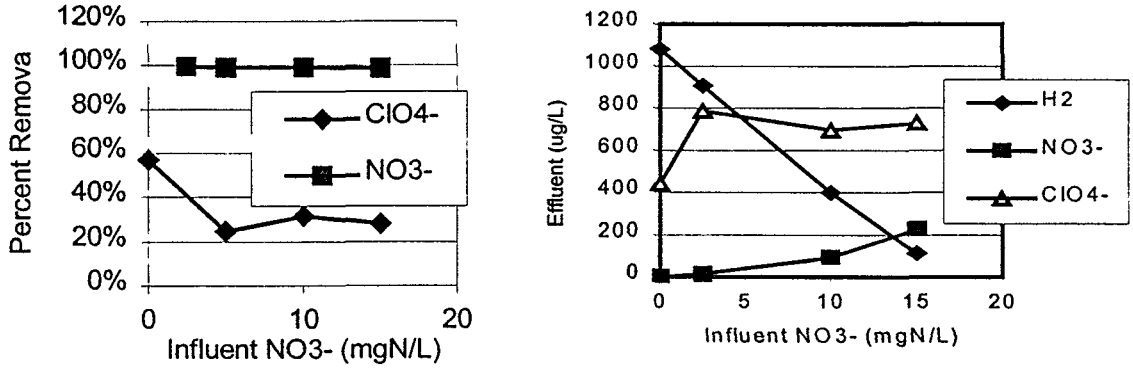


Figure 4
Short Term Experiment 3 – Influent Nitrate is the Variable

Discussion

During the Screening Experiment, perchlorate removal improved from around 40 percent initially to above 95 percent. This suggests that the reactor conditions were selective for perchlorate-reducing bacteria and that over time the reactor was enriched with perchlorate reducers. During development of the steady-state for the Mechanisms Experiments, which included the same conditions as for the Screening Experiment but with a steady-state dissolved hydrogen concentration of only 70 µg/L, similar improvements of perchlorate reduction were not

observed over time. Rather, the perchlorate removal remained at around 15 percent. However, in the hydrogen short-term experiment, the removal efficiency increased with increasing dissolved hydrogen. When the hydrogen was increased to above 0.3 mg/L, the perchlorate removal efficiency increased to 35 percent, similar to the initial removal of the Screening Experiment prior to adaptation to perchlorate. This suggests that high-hydrogen environments may provide a competitive advantage to perchlorate reducers.

During the short-term experiments, up to 22 mg/L of effluent perchlorate did not have any effect on the nitrate reduction efficiency. However, even very low concentrations of nitrate (as low as 0.01 mgN/L) limited perchlorate reduction to about 30 percent, although this reduction did not decrease as the effluent nitrate concentration increased from 0.014 to 0.23 mg/L. Excluding nitrate entirely from the influent increased perchlorate reduction to 57 percent. No accumulation of nitrite or chlorite was observed in any experiment, which suggests that the first step (nitrate to nitrite or perchlorate to chlorate) were rate limiting.

Perchlorate reduction never ceased completely in any of the experiments. This suggests that either there are some species within the biofilm that are not subject to nitrate inhibition, such as HAP-1 (Wallace et al., 1996), or that nitrate did not penetrate into the deeper portions of biofilm, creating a zone where perchlorate reduction occurred free of nitrate inhibition. Inner portions of the biofilm also have higher hydrogen concentrations than the bulk liquid, which, as discussed previously, may be advantageous for perchlorate reduction. It is also possible that some perchlorate reduction was due to nitrate reductase activity. Nitrate reductase is known to reduce perchlorate and chlorate to chlorite. However, no accumulation of chlorite was observed in any of the experiments, and chloride accumulated in amounts approximately stoichiometric to perchlorate disappearance.

Conclusions

The Screening Experiment demonstrated that autohydrogenotrophic reduction has very good potential to be an efficient treatment system for waters with nitrate and low levels of perchlorate. The Mechanisms Experiments identified some important interactions of hydrogen, nitrate, and perchlorate concentrations on perchlorate reduction. Further work is underway to acertain other mechanisms affecting perchlorate reduction, to determine reduction kinetics, and to design a practical treatment system.

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